A METHOD FOR THE CULTIVATION OF BLASTOCYSTIS

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Quite a good deal of uncertainty has existed as to the true nature of blastocystis. Prowazek (1904) and Ucke (1907) first described this organism as a cyst of trichomonas. Alexeieff (1911) was the first to describe blastocystis as a distinct organism of a vegetable nature. Others have described this organism and discussed the possibility of its being a cyst of trichomonas or other intestinal parasite, or some degeneration form of these parasites.

Bohne and Prowazek (1908), Benson (1909), Wenyon (1910), Prowazek (1911), Brumpt (1912), Chatton and Lalung Bonaire (1912), Macfie (1915), Swellengrebel (1917), Chatton (1917), have all given various descriptions of this organism. Wenyon (1920) gives a very comprehensive review and digest of the subject. Parts of his article are quoted below:—

'What then is the blastocystis which occurs so commonly in the human intestine and that of other animals? Prowazek (1911) maintained that they were cysts of trichomonas, but there is no evidence to support this view. Swellengrebel (1917) has suggested that they are degenerate forms of various intestinal protozoa, while Jepps and Dobell (1918) have noted that certain degenerate forms of *Dientamoeba fragilis* resemble dead blastocystis. I myself have, for want of evidence to the contrary, always regarded them as of a vegetable nature, and this may be the case in spite of the resemblance to the cysts of *Prowazekella lacertae*. Swellengrebel's conclusion is that blastocystis "is not the name of a zoological genus but of a peculiar form of degeneration to which representatives of different genera of intestinal protozoa may be liable."' Wenyon concludes, 'It is evident, therefore, that there is a difference of opinion as to the true nature of blastocystis, and we must await further information.'

The writer has had under observation for the past few years a case of balantidial infection, whose stools are loaded with blastocystis as well as balantidia. While attempting to cultivate the balantidia, the blastocystis was found to multiply readily on the medium used. Since then numerous cultures from different individuals have been made. This work was begun in 1919, and the first successful culture made in September of that year. The universal occurrence of this organism may be noted from the articles by Lynch (1917) in the United States, Wenyon and O'Connor (1917) in Egypt, Haughwout (1918) in the Philippines, and Maplestone (1921) in Australia.

METHOD

The culture medium used in this work is very simple, being made up of human blood serum and 0·5 per cent. of sodium chloride solution. Various concentrations of the serum in salt solution have been tried. The most favourable strength has been 10 per cent. Walker (1913) found 0·5 per cent. salt solution to be the proper tonicity for *Balantidium coli*, and this concentration has been used in the work with blastocystis. The salt solution is sterilized in the autoclav and the serum added after inactivation at 55° C. for one half-hour. The pooled serum of several individuals has been used instead of that from a single individual, although no work has been done to show whether or not one serum may be inhibitory while another is favourable to the growth of blastocystis. The sera of animals other than man have not been tried. This medium is faintly alkaline to litmus. The medium is distributed in narrow test tubes in quantity sufficient to give a column of fluid at least 100 mm. high.

No growth takes place at the surface of the tube, and the parasites multiply best at the lower portion of the tube, evidently needing little free oxygen for their growth.

In making the initial inoculation, a small portion of stool or an emulsion of faeces in salt solution is placed at the bottom of the tube containing the culture medium. The culture is incubated at 37° C. for twenty-four to forty-eight hours and then examined for blastocystis. It is best to examine early cultures every twenty-four

hours in order to note the degree of growth of blastocystis and to make transfers before bacterial contamination is too heavy. If good growth is obtained after twenty-four hours, a transplant is then made into fresh culture medium, using the sediment in the original tube. When the organisms have established themselves in the new medium, it is best to make transfers every forty-eight hours. Cultures seventy-two hours old, or older, will hardly ever give good growth in succeeding transfers. As stated above, the optimum growth takes place in the lower portion of the tube. In making all subsequent transfers the sediment from the cultures is used, and is introduced into the bottom of the tubes of the fresh medium. Using this method, the writer has carried blastocystis through more than twenty-five sub-cultures, covering a period of about fifty days. Cultures have been abandoned, usually because of pressure of other work or because of accident or carelessness in making transfers, although they often die from bacterial overgrowth. With proper care a culture can probably be carried on indefinitely. The writer has made no attempt to classify different strains of blastocystis though the gross appearance of organisms from different individuals would suggest strongly that several strains of blastocystis have been encountered. Nor has any attempt been made to trace out a developmental cycle for blastocystis. This task is properly left to one proficient in systematic mycology.

In cultures, budding, as described by Alexeieff, and binary division are the methods of multiplication exhibited by this organism; both of these processes have been observed in all cultures. In the examination of hundreds of preparations the so-called multiple division form of Alexeieff, pictured by Wenyon and O'Connor (1917), has been encountered on only two occasions. This form would seem to be some freak in the development of the organism rather than a true developmental phase. In cultures, as in the intestinal contents, a great variation in the size of the organisms is observed. It may be said with a reasonable degree of certainty that the large vacuolated forms are the result of degeneration, as these forms are commonly found in old cultures, and successful transfers cannot be made from cultures made up of these forms. No flagellate forms, and no forms showing amoeboid or other motion have been encountered. Blastocystis retains its original form after twenty-five successive transfers

in artificial culture medium, and no forms were seen in the twenty-fifth transfer that were not seen in the first.

This work is submitted as proof that blastocystis is a distinct zoological genus, and not a cyst of protozoa nor a form assumed by protozoa undergoing degeneration.

REFERENCES

Alexieff, A. (1911). Sur les 'Kystes de Trichomonas intestinalis' dans l'intestin des Batraciens. *Bull. Sci. France et Belgique*, Vol. XLIV, p. 333.

——— (1911). Sur la nature des formations dites 'Kystes de Trichomonas intestinalis.' *C. R. Soc. Biol.*, Vol. LXXI, p. 296.

Benson (1910). Untersuchungen über Trichomonas intestinalis und vaginalis des Menschen. *Arch. f. Protistenk.*, Vol. XVIII, p. 116.

Bohne und Prowazek, S. (1908). Zur Frage der Flagellatendysenterie. *Arch. f. Protistenk.*, Vol. XII, p. 1.

Brumpt, E. (1912). Colite à *Tetramitus mesnili* (Wenyon 1910) et colite à *Trichomonas intestinalis* Leuckart 1879. *Blastocystis hominis*, n. sp. et formes voisines. *Bull. Soc. Path. Exot.*, Vol. V, p. 725.

Chatton, E. (1917). Les 'Blastocystis' stades du cycle évolutif de flagelles intestinaux. *C. R. Soc. Biol.*, Vol. LXXX, p. 555.

Chatton, E. et Lalung-Bonnaire (1912). Amibe limax (*Vahlkampfia* n. gen.) dans l'intestin humain. Son importance pour l'interprétation des amibes de culture. *Bull. Soc. Path. Exot.*, Vol. V, p. 135.

Haughwout, F. G. (1918). The tissue invasive powers of the flagellated and ciliated protozoa, with especial reference to *Trichomonas intestinalis*. A critical review. *Philippine Journ. of Science*, Sec. B, Vol. XIII, p. 217.

Jepps, M. W., and Dobell, C. (1918). *Dientamoeba fragilis* n.g., n. sp., a new intestinal amoeba from man. *Parasitology*, Vol. X, p. 352.

Lynch, K. M. (1917). *Blastocystis hominis*; its characteristics and its prevalence in intestinal content and faeces in South Carolina. *Journal of Bacteriology*, Vol. II, p. 369.

Macfie, J. W. S. (1915). A case of dysentery in a monkey in which amoebae and spirochaetes were found. *Ann. Trop. Med. and Parasit.*, Vol. IX, p. 507.

Maplestone, P. A. (1921). Human Intestinal Protozoa in North Queensland. *Ann. Trop. Med. and Parasit.*, Vol. XIV, p. 283.

Prowazek, S. (1904). Untersuchungen über einige parasitische Flagellaten. *Arb. a. d. Kais. Gesundheitsamte*, Vol. XXI, p. 1.

——— (1911). Zur Kenntnis der Flagellaten des Darmtraktus. *Arch. fur Protistenk.*, Vol. XXIII, p. 96.

Swellengrebel, N. H. (1917). Observations on *Blastocystis hominis*. *Parasitology*, Vol. IX, p. 451.

Walker, E. L. (1913). Quantitative determination of the balantidicidal activity of certain drugs and chemicals as a basis for treatment of infections with *Balantidium coli*. *Philippine Journ. of Science*, Sec. B, Vol. VIII, p. 1.

Wenyon, C. M. (1910). A new flagellate, '*Macrostoma mesnili*' n. sp., from the human intestine, with some remarks on the supposed cysts of '*Trichomonas*.' *Parasitology*, Vol. III, p. 210.

——— (1920). Observations on the intestinal protozoa of three Egyptian lizards, with a note on a cell-invading fungus. *Parasitology*, Vol. XII, p. 350.

Wenyon, C. M., and O'Connor, F. W. (1917). Human intestinal protozoa in the Near East. *Wellcome Bureau of Scientific Research*, London.